INTRODUCTION

Plant hormones are signal molecules that act as chemical messengers to control plant growth and development. Aside from their role in plant response to changes in environmental conditions, hormones are also the principal agents that regulate expression of the intrinsic genetic potential of plants. A phytohormone is an organic substance synthesized in defined organs of the plant that can be translocated to other sites, where it triggers specific biochemical, physiological, and morphological responses. However, phytohormones are also active in the tissues where they are produced. The commonly recognized classes of phytohormones, regarded as the "classical five", are: the auxins; gibberellins; cytokinins; abscisic acid; and ethylene. Some microorganisms also produce phytohormones.

Gibberellins (GAs) are a large group of important diterpenoid acids among commercial phytohormones (Martin et al. 2000). Gibberellins are tetracyclic diterpenoid acids those are involved in a number of developmental and physiological processes in plants (Crozier et al. 2000; Davies 1995). These processes include seed germination, seedling emergence, stem and leaf growth, floral induction and flower and fruit growth (King and Evans 2003; Pharis and King 1985; Sponsel 2003). Gibberellins are also implicated in promotion of root growth, root hair abundance, and inhibition of floral bud differentiation in woody angiosperms, regulation of vegetative and reproductive bud dormancy and delay of senescence in many organs of a range of plant species (Tanimoto 1987; Bottini and Luna 1993; Fulchieri et al. 1993; Reinoso et al. 2002).

Considering the numerous effects of gibberellins, it seems logical that they would be used in commercial applications. Their major uses are

- Management of fruit crops - most seedless table grapes are now grown with the application of GA_3, inhibiting senescence of citrus fruit maintaining the rind in better condition; controlling skin disorder in "golden delicious" apples;
- Production of ornamental plants - inducing to flower either earlier than usual, or in off-seasons. Sporadic flowering in some plants is often a problem with plant breeders, but may be ameliorated with GA applications;
- Malting of barley: 2-3 days may be saved by the addition of 25-500 µg of GA_3 for each kg of barley.
- Extension of sugarcane: increase in grown and sugar yield.

(Martin 1983; Taiz & Zeiger 1991; Mander 2003)

Gibberellins are produced not only by higher plants and fungi (MacMillan 2002) but also by bacteria (Atzorn et al. 1988; Bottini et al. 1989; Janzen et al. 1992; Bastián et al. 1998; Gutiérrez-Mañero et al. 2001). In fungi and bacteria, there is no known role for gibberellins; rather they seem to be secondary metabolites that may play a role as signaling factors towards the host plant. A number of micro-organisms have been reported to produce GA_3 and GA like substances. To date, 136 GAs from higher plants (128 species), 28 GAs from fungi (7 species), and only 4 GAs (GA_1, GA_3, GA_4, and GA_{20}) from bacteria (7 species) have been identified (MacMillan, 2002).

Gibberellic acid (GA_3) is the main product of gibberellins in fungi and bacteria (Bruckner and Blecschmidt, 1991). GA_3 is a high value industrially important biochemical selling at $ 27-36/g (United State Currency) in the international market, depending upon its purity and potency. Therefore, its use at present is limited to high premium crops. In the recent past gibberellin has been used to increase the rice panicle length and to produce droopy leaves for producing more hybrid seeds (Hanqiao et al. 1997). As the demand of gibberellin is increasing, the research and developmental efforts on the production of GAs has been intensified.

Research was undertaken to screen the potential gibberellin producing microorganisms and maximize the gibberellin yield. The primary objectives of the research were as follows:

OBJECTIVES

- Screening of the microorganisms for gibberellin production and selection of the most potential ones.
- Characterization of gibberellin producing isolates.
- Optimization of Physico-chemical parameters for maximum gibberellin production.
- Production of Gibberellins with the optimized medium & process parameters and analysis of produced gibberellin by using one or more of the following methods
    - HPTLC
    - GC-MS
    - HPLC
- Microbial broth extracts would be experimented for plant growth promotion with pot experiments.

RESEARCH SUMMARY

SAMPLE COLLECTION AND ISOLATION

Rhizosphere and rhizoplane samples were collected from the agriculture fields in the vicinity of Surat, Gujarat, India. For isolation of rhizobacteria and rhizosphere fungi, plants were carefully dug out without damaging the roots along with the adherent soil and were brought to the laboratory in polythene bags. The soil particles loosely adhering to the roots were gently teased out and used for isolation of rhizobacteria and rhizosphere fungi. Soil particles adhering to the roots were used for isolation of rhizoplane bacteria and fungi.

A total of about 59 bacteria designated as NPB 1-59 and 12 fungi designated NPF 1-12, were isolated from rhizosphere and rhizoplane samples of four local crop plants as Rice (*Oryza sativa*), Sugarcane (*Saccharum officinarum*), Wheat (*Triticum aestivum*) and Chana (*Cicer arietinum*).

SCREENING OF THE ISOLATES FOR GIBBERELLIN PRODUCTION

A total of 59 rhizobacteria and 12 rhizosphere fungi obtained were screened for their ability to produce gibberellin. For rhizobacteria the Nutrient medium was used and for rhizosphere fungi Saboroud's medium was used. 48 h old growth of bacteria was centrifuged at 10,000 rpm for 15 - 20 min and 8 days old growth of fungi was filtered through Whatman No. 42 filter paper. The culture supernatants and culture filtrates were used to detect GA_3 production by spectrophotometric method.

Out of 59 bacteria and 12 fungi, 41 bacteria and 8 fungi reported as GA_3 producer by spectrophotometric method. Amongst bacterial isolates NPB 13 & NPB 20 produced 88.04 & 93.93 mg/L of gibberellin and amongst fungal isolates NPF 7 produced 184.11 mg/L of GA_3, which was significantly more than the other isolates.

IDENTIFICATION OF THE ISOLATES

Bacterial isolates NPB 13 & NPB 20 were identified as *Bacillus cereus* and *Pseudomonas monteilii* by biochemical as well as 16s rDNA sequencing and fungi NPF 7 was identified as *Aspergillus flavus* on the basis of their colony & morphological characteristics and 18s rDNA sequencing.

OPTIMIZATION OF PHYSICO-CHEMICAL PARAMETERS FOR MAXIMUM GIBBERELLIN PRODUCTION

Influence of Various Environmental Conditions on Growth and Gibberellin Production

The effect of Inoculum sizes (10^4 - 10^9 cells or spores/ml), temperatures (20°C - 40°C), pH (4 - 8) and mode of cultivation (static and shaking) on growth and gibberellin production were examined, from 24 to 72 h for bacteria and from 3rd day onwards at every alternate day up to 11 days for fungi.

The GA_3 yield was maximum (394.15 & 318.24 mg/l) after 60 h of incubation at 6.0 pH & 30°C temperature on a rotary shaker with the inoculum size of 10^7 cells/ml of *Pseudomonas monteilii & Bacillus cereus* respectively, whereas in case of *Aspergillus flavus*, the GA_3 yield was maximum (590.18 mg/l) after 9 days of incubation at 5.0 pH & 30°C temperature on a rotary shaker with the inoculum size of 10^8 spores/ml.

Effect of sugars on Gibberellin Production

The effect of 3 sugars, glucose, maltose and sucrose, revealed that all of the sugars increased GA_3 production for *Pseudomonas monteilii & Bacillus cereus*. In case of *Aspergillus flavus*, glucose and sucrose increased the gibberellin production.

Effect of nitrogen sources on Gibberellin Production

The effect of 3 nitrogen sources, Ammonium chloride, Sodium nitrate and Urea, revealed that sodium nitrate increased GA_3 production for all isolates. Ammonium chloride had inhibitory effect on all isolates while urea had no effect on GA_3 production.

Determination of carbon-nitrogen ratio for Gibberellin Production

Determination of carbon-nitrogen ratio is very important for the production of secondary metabolites by microbes (Cacciari *et al*., 1989). For this purpose, the experiment was carried out in two ways for determination of suitable carbon-nitrogen ratio for GA_3 production. In first experiment carbon source (Glucose or Sucrose) was kept at constant (100 mM) concentration and nitrogen source ($NaNO_3$) was added to nutrient broth at concentration of 10 to 40 mM. In the second experiment, nitrogen source ($NaNO_3$) was kept at fixed concentration (10 mM) and carbon source (Glucose or Sucrose) was added to nutrient broth at concentration of 50 to 200 mM. From the obtained results optimum carbon-nitrogen ratio was determined to be 100:10 mM.

Effect of mevalonic acid on Gibberellin Production

Stimulation of gibberellin production in submerged culture using a commonly known precursor, mevalonic acid (MVA), was investigated in terms of growth and gibberellic acid production. Increasing concentrations of MVA up to 60 µM enhanced product and growth yields. Above this amount, gibberellic acid yields and growth were gradually decreased.

PRODUCTION & CHEMICAL CONFIRMATION OF GIBBERELLINS WITH THE OPTIMIZED MEDIUM AND PROCESS PARAMETERS IN LABORATORY

Production of gibberellin was carried out with the optimized medium and process parameters in laboratory, which reveals that all three isolates produced gibberellins in significant amount which was further confirmed by HPLC method. HPLC analysis suggested that all the three isolates are able to produce GA_3 as well GA_4 which are considered as bioactive forms of gibberellins.

PLANT GROWTH PROMOTING CAPACITY OF MICROBIAL ISOLATES

The microbial isolates were bioassayed on wheat and chana for its growth promoting capacity. An experimental design contains one negative control, one positive control and three tests, where negative control and tests seeds treated with 100 ppm uniconazole (natural gibberellin inhibitor). These pretreated seeds were transferred to prelabeled respective pots containing sterilized soil mixture. Pots were irrigated with double distilled water (for negative and positive controls) or appropriate microbial broth extracts with desired dilution (for tests) and incubated at 30 ± 2°C for 12 days.

The microbial broth suspension of NPB13, NPB20 & NPF7 significantly promoted growth of wheat and chana seedlings. In both crops, seed germination, the root length, and shoot length parameters significantly promoted compared to positive control.

IMPLICATION OF THE RESEARCH

Commercial production of GA_3 and related gibberellins not only helps in boosting agriculture but also various industrial processes. In presented study an attempt has been made to isolate the most potential Gibberellin producing microorganisms. The culture medium and conditions were optimized for gibberellin producing isolates in order to increase gibberellic acid production. This work proved that with careful selection of nutritional and physical factors, the yield of GA_3 can be enhanced. In the presented study, the increase in gibberellin production was almost 5 times which will be helpful to produce GAs in higher yields at economic costs.

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